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Supplemental Data

Mitochondrial Fusion Protects against Neurodegeneration in the Cerebellum

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Supplemental Tables and Figures

Table S1. Lethality of Meox2-Cre/*Mfn*^{loxP} mice

Mfn1	Mfn2	Age of Lethality
+/+	+/+	> 1 year [a]
+/-	+/+	> 1 year [a]
+/+	+/-	> 1 year [a]
+/-	+/-	> 1 year [a]
-/-	+/+	> 1 year [b]
-/-	+/-	P0 [c]
+/+	-/-	P1-P17 [d]
+/-	-/-	P1-P17 [c]
-/-	-/-	P0 [c]

[a] These mice show no abnormalities and have normal life spans.
[b] Generated from Meox2-Cre, *Mfn1*^{null}/+ X *Mfn1*^{loxP}/*Mfn1*^{loxP} matings. 12 litters examined. Four mice were monitored till 9.5-13 months of age. These mice showed no abnormalities and presumably have normal lifespans.
[c] Generated from Meox2-Cre, *Mfn1*^{null}/+, *Mfn2*^{null}/+ X *Mfn1*^{loxP}/*Mfn1*^{loxP}, *Mfn2*^{loxP}/*Mfn2*^{loxP} matings. 10 litters examined.
[d] Generated from Meox2-Cre, *Mfn2*^{null}/+ X *Mfn2*^{loxP}/*Mfn2*^{loxP} matings. 20 litters examined.

Table S2. L7-Cre/*Mfn2*^{loxP} Rotarod Test

Age (weeks)	Control (sec)	Mutant (sec)
8.5	144 ± 7	134 ± 12
9.5	137 ± 19	125 ± 19
10.5	132 ± 36	117 ± 23
11.5	143 ± 31	107 ± 14
12.5	142 ± 19	82 ± 23
13.5	145 ± 17	96 ± 43

Cohorts of 5 wild-type and 6 mutant mice were tested weekly. Each mouse underwent three trials, and the times given represent the average of all trials ± standard deviation.

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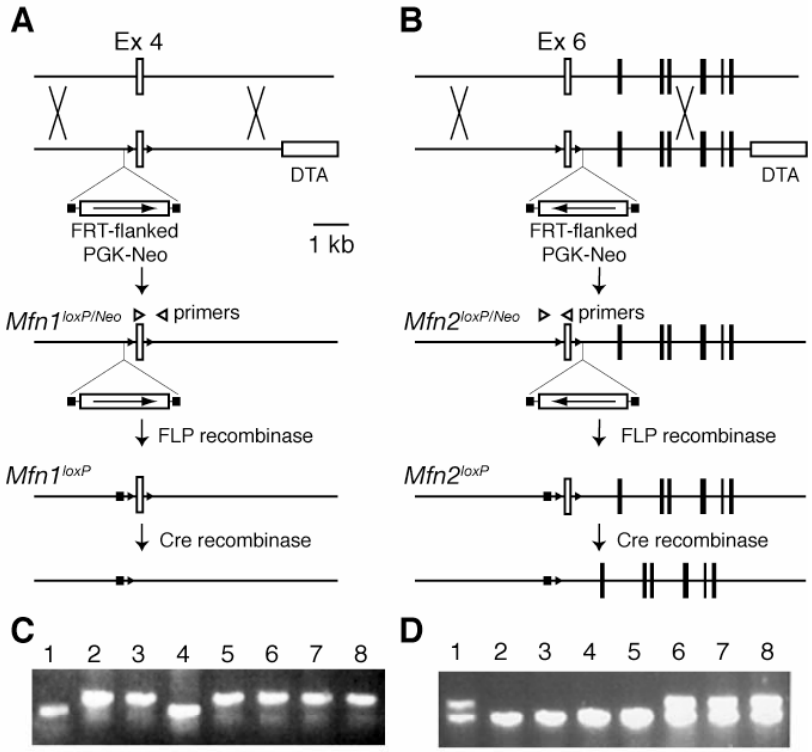
Figure S1



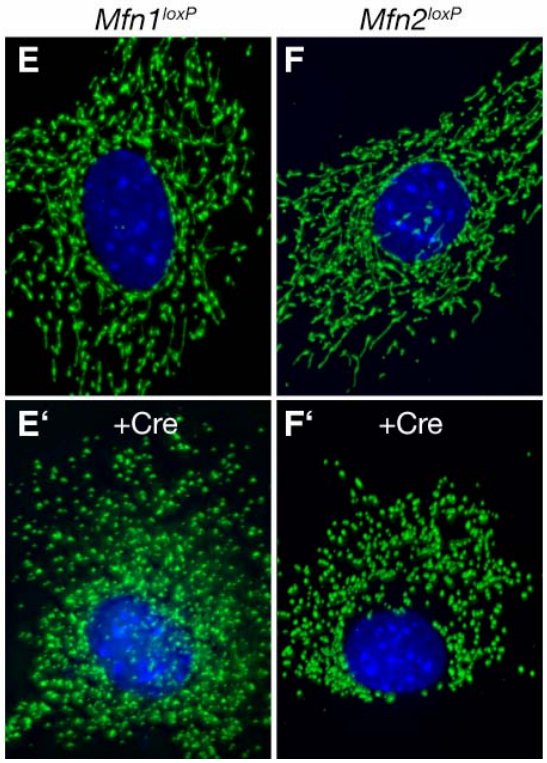


Figure S1. Conditional *Mfn1* and *Mfn2* Knockouts

(A and B) Schematic diagram of conditional targeting constructs for *Mfn1* (A) and *Mfn2* (B) and their homologous recombination into the endogenous loci. In each case, two *loxP* recombination sites flank the exon encoding the canonical G-1 GTPase motif. When exposed to Cre recombinase, excision of this critical exon and a frame-shift occur, thus preventing formation of any functional protein. Symbols: black squares, FRT sites; black arrowheads, *loxP* sites; white arrowheads, PCR primers; DTA, diptheria toxin.

(C and D) PCR genotyping of $Mfn1^{loxP}$ (C) and $Mfn2^{loxP}$ (D) mice. Primers used are shown in (A and B). Upper bands contain *loxP* site, lower bands are wild-type.

(E and E') Mitochondrial morphology in $Mfn1^{loxP}$ MEFs in the absence (E) or presence of Cre (E'). Mitochondria were visualized with mitochondrially targeted EGFP (green), and nuclei were counterstained with DAPI (blue).

(F and F') Same as (E and E') except with $Mfn2^{loxP}$ MEFs.

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Figure S2

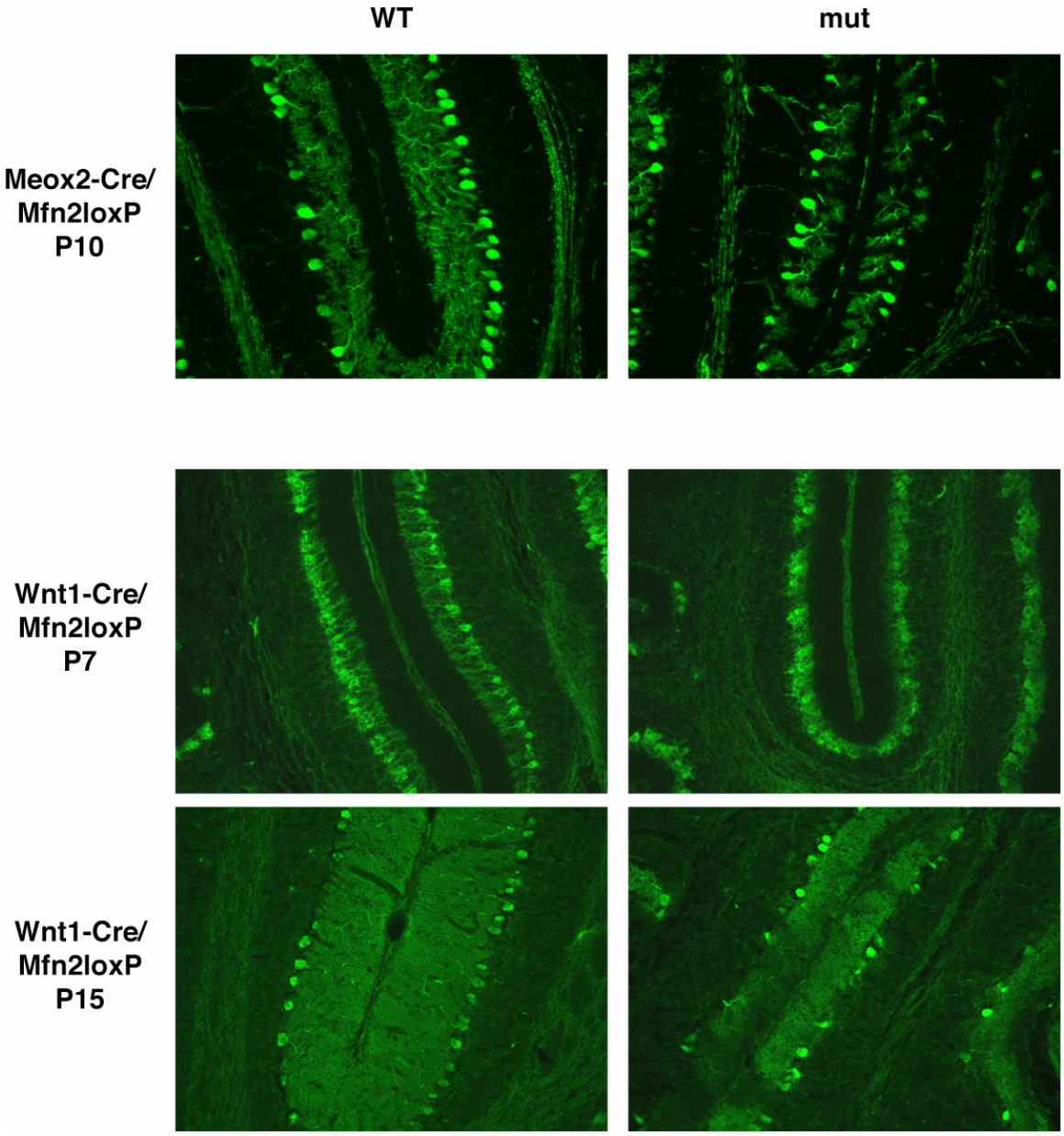

Figure S2. Degeneration of PCs in Meox2-Cre/*Mfn2*^{loxP} and Wnt1-Cre/*Mfn2*^{loxP} Mice

Anti-calbindin IF at ages indicated. Meox2-Cre and Wnt1-Cre mutants both show similar phenotypes to En1-Cre/*Mfn2*^{loxP} mice, where PC dendritic outgrowth is stunted, and Purkinje cell loss increases with age.

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Figure S3

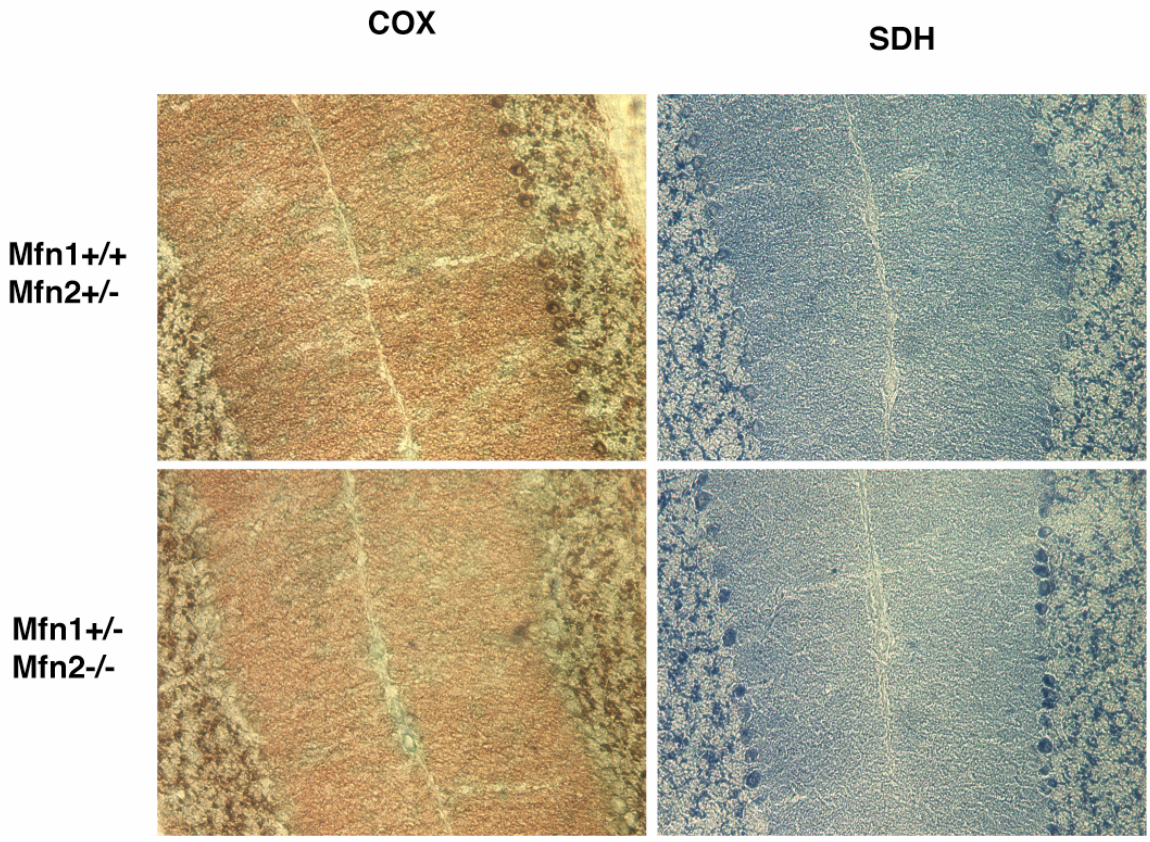

Figure S3. Electron Transport Compromise in Young L7-Cre/*Mfn2*^{loxP} Mice
COX and SDH staining of 6 week-old cerebella. PCs without Mfn2 (lower panels) show decreased COX activity and increased SDH activity prior to Purkinje cell loss. A littermate control (upper panels) demonstrates moderate staining of both complexes.

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Figure S4

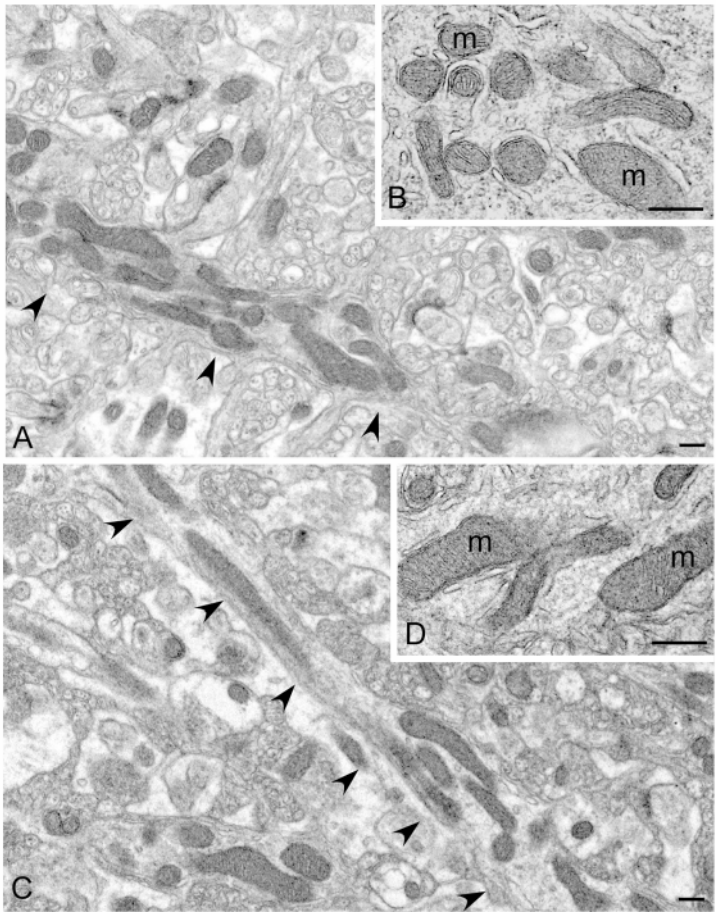

Figure S4. Ultrastructure of Mfn1-Deficient PCs
(A and B) Representative EM of 7 week-old Meox2-Cre/*Mfn1*^{loxP} cerebellum (all cells *Mfn1*-/-). Mitochondria (m) are elongated, have well-folded cristae, and fill dendritic tracts (arrowheads).
(C and D) Analogous images to (A and B) for 9 week-old L7-Cre mouse with PCs of genotype *Mfn1*-/-, *Mfn2*+/-. Again, mitochondrial morphology and distribution is normal.
Scale bars indicate 0.5 μm.